R: REDACTED MATERIAL

BIOASSAY, CARCINOGENESIS AND TISSUE CULTURE

No. 535

Application for Cancer Funds
Cancer Research Coordinating Committee
University of California

COMMITTEE
Dr. Little, Chm.
Dr. Andervont
Dr. Reimann

I. New application (Prior grant for related work)

II. Faculty Investigator

Prof. J.F. Thomas
Civil Engineering

Dr. C.S. Chen
Assistant Research Microbiologist
Sanitary Engineering Research Laboratory

III. Prof. P.H. McGauhey
Director SERL

IV. The Development of a Microbiological Screening System for Assay of Air Borne Carcinogens.

V. Amount Requested for 1955-1956

$6800.00

VI. Budget:

REDACTED

Supplies & Expense	
Chemicals, glassware & expendable supplies	700.00
	$6800.00

VII. Explanation of the Project

The air pollution research laboratory in Sanitary Engineering has been concerned over a period of 12 years with the development of sampling and analytical techniques for atmospheric pollutants. Numerous publications have been made as the result of this work.

Related to the analytical work is a growing awareness that a large number of the identified atmospheric pollutants may be classed as carcinogenic. It is possible to separate hundreds of unknown compounds in relatively pure form from the atmosphere but no identification has yet been made other than noting by chemical and chromatographic behavior that there are structural similarities between the carcinogens and the unknown compounds. (For example, perylene or pentacene may be classed as non-carcinogens, yet falling

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in the same hydrocarbon class as benz(a)pyrene, a carcinogen - all are atmospheric pollutants.) Further, the atmospheric pollutants are collected only in such minute quantities that assaying for carcinogenic activity by conventional techniques is impossible.

The original thought was to find a single micro organism that could be added to the list of conventional test animals, such as mice, rabbits, dogs, etc. Coupled with a simple test, with the index for carcinogenic activity being based on induced morphological or biochemical variations compared to the control. The advantages of using micro organisms are (1) simplicity; (2) rapidity; (3) only minute quantities of test material are required; (4) relatively inexpensive; (5) close environmental control; (6) a large number of "test animals" with respect to generations and populations easily maintained and readily available for test purposes.

Bacillus megaterium was the original organism selected using relatively few compounds of known composition for the developmental work. An attempt was made to force the organism to indicate according to established carcinogenic activity of the compounds, by modifying the environment, i.e. those compounds classed as carcinogenic were to cause the organism to behave in a manner quite different from organisms exposed to non-carcinogens or the control. (A paper has been published on this work.) It soon became apparent that a single organism would not satisfy if simplicity and rapidity were to be considered as essential to the developmental objectives.

It does appear feasible to develop a number of simple tests using a variety of organisms. A large number of test compounds have been gathered (list included) and the list is being gradually increased. It is hoped that a sufficient number of individual tests may be developed so that any compound giving, for example, three positive tests out of a possible ten total tests will be at least statistically indicative of carcinogenic activity.

Work is currently being done using (1) Photobacterium phosphoreum (ATCC 11040), (a paper has been prepared for publication covering this work to date); (2) Pasteurella tularensis (ATCC 6223), Lysogenic, Dept. of Bacteriology, University of California, Berkeley; (3) Bacillus megaterium; (4) an "unknown rod" isolate from sewage (A Pseudomonas-like organism found to grow in the presence of 5 percent acetone, allowing for increased solubility of test hydrocarbons). Emphasis is being placed on finding general and specific enhanced or retarded enzyme activities as indices of carcinogenic activity although many morphological and biochemical variations occur simultaneously which are also being given cursory examination for possible future exploratory work. At the suggestion of Dr. DeOme part of the screening should include a simple tissue culture aspect to serve as a bridge relating the findings of these

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atypical tests to the more conventional assay techniques.
(Tentative arrangements have been made to use some facilities at the U.C. Medical Center, S.F. for the tissue culture work.)

Monies are requested to enable the work to continue along the broad exploratory paths now being followed.

VIII. Although the major objective has been to develop a screening technique for assaying carcinogenic activity of minute quantities of material, the studies to date involve work on single cell organisms in a completely controlled environment which looks very promising as leading to an insight to chemical carcinogenesis in higher animals.

IX. Facilities and equipment of the major air pollution projects are available for use by this small auxiliary project. Help is required only for salaries and expendable supplies as indicated in the budget. A proposal is being prepared for submission to the Public Health Service to carry this research as an entity in itself.

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SECTION II -- PRIVILEGED COMMUNICATION

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1. RESEARCH PLAN

A. Introduction and Specific Aims

(1) Description

Mammalian animals are conventionally used to assay carcinogenic activity. These assay techniques are not sensitive enough for use with suspect atmospheric pollutants because only small quantities of test material are available. In recent years there has been an indication that microorganisms sensitive to minute quantities might be used for an alternate assay system. Probably not a single organism but a select group must be used for screening because of different metabolic patterns of the different organisms.

The advantages of a microbial assay system include: 1) simplicity; 2) rapidity; 3) only minute quantities of test material are required; 4) relatively inexpensive; 5) close environmental control; 6) large number of "test animals" easily maintained and readily available for test purposes.

Several microbiological assay techniques have already been developed incorporating growth response, enzyme activity, photodynamic action and induced morphological and cytochemical changes. Each individual test appears promising but each also gives anomolous results with certain compounds.

The general objective of the proposed work is to develop new techniques and improve existing techniques so that each carcinogen will give two or more positive tests using a screening program involving a number of test organisms.

Preliminary work on bacterial enzyme activity has also indicated several parallels to more conventional cancer research, which might eventually give some insight into the mode of carcinogenesis.

(2) Rationale

The basic assumption is that carcinogenic compounds will affect microorganisms differently than structurally similar noncarcinogenic compounds. A variety of isomeric compounds must be included in the test program to be sure the indicating effect is due to carcinogenic activity and not size, molecular weight or other variables of little consequence.

The rationale behind the basic assumption may be summed up briefly in five points (i through v). These are presented in outline form and in expanded detail under the pertinent literature references in Section 2.C.

Possible indicating effects of carcinogens on bacterial cells:

1) Indirect effects: (i) In photodynamic action, the carcinogens serve as sensitizing agents. Recognized at cellular level--death of the cells. (ii) The carcinogens change cellular surface to permit or stimulate variants to grow. Recognized at cellular level--formation of heterogenic population or predominant variants.

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2) Direct effects: (iii) The carcinogens change gene(s) in a cell by interacting with DNA. Recognized at cellular level--formation of mutants and heterogenic population. Recognized at subcellular level--change in metabolic patterns and enzyme deletion. (iv) The carcinogens interact with enzymes and non-enzyme proteins. Recognized at subcellular level--change in metabolic pattern and enzyme deletion. (v) The carcinogens participate in induced enzyme formation (adaptive enzymes). Recognized at subcellular level--inducing synthesis of TPNH-dependent enzymes.

(3) Specific Aims

The research to be undertaken will be directed toward the establishment of microbial indicators of carcinogenic arene type hydrocarbons using induced atypical physiological and biochemical responses with special emphasis on enzymatic activities both at intact and subcellular levels. An outline of the study plan follows with details being given under methods of procedure (Section I.B.).

1) To evaluate the growth rate of microorganisms as indicator for carcinogenic effect with special references to stimulatory effect on facultative, anaerobic organisms and inhibitory effect on aerobic organisms.

2) To investigate the biochemical aspects of carcinogenic effect upon the microbial system by comparing the following characteristics in cell treated with carcinogens and with non-carcinogens.

a) Intact cellular level
   i) Oxygen uptake using Warburg manometric technique
   ii) Rate in utilization of glucose--quantitative wet chemical analysis with respect to time
   iii) Rate in uptake of inorganic phosphate--quantitative wet chemical analysis with respect to time

b) Subcellular level
   i) Activities of some enzymes involving carbohydrate metabolisms --classical methods will be followed
   ii) Activities of enzymes containing active sulfhydryl group
   iii) Activities of TPNH-dependent enzymes
   iv) Isolation and characterization of metabolites using chromatography

3) To select the enzymes highly associated with tumor cells in animals and to evaluate their activities in microbial system treated with carcinogens.

4) To evaluate and determine photodynamic activities in microbial system.

5) To examine the effect of the hydrocarbons on morphological and cytochemical aspects of the bacterial cells.

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B. Methods of Procedure (References refer to Pertinent Literature References listed under section 2.C.; see p. 14)

Approximately 50 compounds will be used in the development and evaluation techniques. Primarily arene type hydrocarbons associated with polluted atmospheres will be used; a list is included in Table I (these compounds are currently available, being used in conjunction with other research currently in progress in this laboratory related to air pollution). A variety of sizes, shape, number of condensed rings and molecular weights are included among the compounds including a number of carcinogenic and non-carcinogenic isomers.

The species of bacteria to be studies include 1) Photobacterium phosphoreum (ATCC 11040), 2) Pasteurella tularensis (ATCC 6223), 3) Bacillus megaterium (lysogenic, Department of Bacteriology, University of California), 4) an "unknown rod" isolate (Pseudomonas-like organism). This organism, isolated from sewage, was found to grow in the presence of 5 percent acetone media. (Incorporation of the acetone is to increase the solubility of testing hydrocarbons.) Identification of this latter organism will be made.

I) Growth Aspects. Liquid media will be used for studying the effects of incorporated hydrocarbons on growth. The growth will be determined turbidimetrically using wavelengths of 600 mμ for medium containing peptone and 400 mμ for colorless medium. Hydrocarbons will be added as an acetone solution. With exception of the "unknown rod" all the acetone will be removed from the medium in vacuum oven at 50°C. Individual compound will be tested first and later various combinations of carcinogens and non-carcinogens will be made to see any effect on growth by interaction between or among the hydrocarbons. All the results will be expressed in per cent growth based on the control sample. Triplicate tubes for each treatment will be made.

Since 100 per cent differentiation of carcinogens from non-carcinogens may seem impossible, therefore two or more assay organisms may have to be teamed up for a systematic screening of the carcinogens.

II) Biochemical Aspects. (A) Enzymatic studies: Assays will include both intact cells and cell-free systems.

a) Intact cells: All the assaying bacterial cells preincubated with or without the testing hydrocarbon will be harvested and the test will be made in the presence of the hydrocarbon. Specific activities of each enzyme assay will be compared between cells treated with carcinogens and with non-carcinogens. Specific activities will be expressed as: activity per unit number of viable cells or activity per unit dry weight. Viable counts will be made by diluting cell suspension to a proper concentration and the dilute suspensions will then be plated out in triplicate with suitable agar and incubated at proper temperature for certain period before counting. The dry weight of a cell suspension will be made by drying aliquots of the cell suspension in 105°C oven for overnight.

b) Cell free preparation: Bacterial cells preincubated with or without the testing hydrocarbons will be harvested and the cell free enzyme will be prepared by sonic treatment, grinding with alumina oxide or glass. Testing hydrocarbons will

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TABLE I. LIST OF AVAILABLE TESTING HYDROCARBONS

1. Anthracene
2. 2-methylanthracene
3. 9-methylanthracene
4. 9,10-dimethylanthracene
5. Benz(a)anthracene
6. 3'-methylbenz(a)anthracene
7. 4'-methylbenz(a)anthracene
8. 3-methylbenz(a)anthracene
9. 4-methylbenz(a)anthracene
10. 5-methylbenz(a)anthracene
11. 6-methylbenz(a)anthracene
12. 7-methylbenz(a)anthracene
13. 8-methylbenz(a)anthracene
14. 9-methylbenz(a)anthracene
15. 10-methylbenz(a)anthracene
16. 9,10-dimethylbenz(a)anthracene
17. 7,12-dimethylbenz(a)anthracene
18. 2-phenylanthracene
19. 9,10-diphenylanthracene
20. Dibenz(a,c)anthracene
21. Dibenz(a,h)anthracene
22. Dibenz(a,j)anthracene
23. Benz(b)anthracene (Naphthacene)
24. Benzo(b)naphthacene (pentacene)
25. Benzo(a)pentacene
26. Dibenz(de,kl)anthracene (perylene)
27. Benzo(ghi)perylene
28. Dibenzo(ghi,pqr)perylene (colonene)
29. 20-methylcholanthrene
30. pheranthrene
31. chrysene (benzo(a)phenanthrene)
32. Benzo(a)chrysene (piecene)
33. Dibenzo(b,k)chrysene
34. Dibenzo(def,mno)chrysene
35. Benzo(l)phenanthrene
36. Benzo(c)phenanthrene
37. Dibenzo(a,i)phenanthrene
38. Pyrene(benzo(def)phenanthrene)
39. Benzo(e)pyrene
40. Benzo(a)pyrene
41. 5-methylbenzo(a)pyrene
42. Dibenzo(a,e)pyrene
43. Dibenzo(e,l)pyrene
44. Dibenzo(a,h)pyrene
45. 5-methyldibenzo(a,h)pyrene
46. 5,10-dimethyldibenzo(a,h)pyrene
47. Fluoranthene
48. Benz(e)acephenanthrylene
49. Benzo(k)fluoranthene
50. 11 H-benz(a)fluorene
51. 11 H-benzo(b)fluorene
52. 2,4,7-trinitro-9-fluorenane
53. 9-fluorenane
54. 5,7-dimethylbenz(a)acridine

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then be mixed with the enzyme for short period so the interaction between the hydrocarbons and the enzyme can take place. With this system, permeability barrier is eliminated. Substrates and other cofactor will be added just before the assay. The specific activity will be expressed as activity per unit dry weight or activity per unit weight of protein. The protein determination will be made using Lowry's[1] Folin-Ciocalten method. Egg albumin will be used for preparing standard curves.

Assay procedures for various enzymes studies on intact and cell-free systems will be made by spectrophotometric and manometric methods. A tentative list of enzymes to be studied includes: 1) Enzymes of carbohydrate metabolisms:[2] glucose-6-phosphatase, fructose-1-6-diphosphatase, phosphoglucomutase, phosphohexose isomerase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate-dehydrogenase (these enzymes are highly associated with animal tumor). 2) Enzymes containing active -SH groups:[3] triose-phosphate dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase. 3) TPNH-dependent enzymes:[3] aldehyde dehydrogenase, benzaldehyde dehydrogenase, 5-dehydroshikimic reductase, glucose-6-phosphate dehydrogenase glutathione reductase, isocitric dehydrogenase, malic enzyme, nitrite reductase. 4) Amino acid enzymes associated with animal tumor:[4] treonine dehydrase, histidase, α-kete glutaric dehydrogenase. 5) Others: Catalase.[5]

(B) Respiration studies: Previous studies of Ph. phosphoreum has shown that carcinogens suppress the respiration of the organism compared to non-carcinogens. The respiration rate was measured by dimming time method. Further tests employing addational test compounds and using manometric technique[6] for measuring oxygen consumption will be made. Cells preincubated with the carcinogens will be used for the assay. Total uptake of oxygen in unit time and in unit dry weight will be expressed as specific activity for comparison.

(C) Uptake of glucose and inorganic phosphate: Glucose is utilized as an energy source, and inorganic phosphate is provided for phosphorylation. Test of the uptake of these two components in the presence of testing hydrocarbons in liquid medium will probably furnish information concerning energy problems. A certain period will be allowed for the uptake of the glucose and the inorganic phosphate; the remaining glucose and inorganic phosphate will be determined so that the net uptake of both can be estimated. The Lowry-Lopez method[7] will be used for the phosphate determination and the "glucostat" method of Worthington Biochemicals, Freehold, for glucose.

(D) Analysis of metabolites: The microorganism will be grown on agar plus testing hydrocarbons in petri dish or Roux cultures bottle. The harvested cell will be washed and solubilized with NaOH or hypochlorite. The metabolized hydrocarbons are released so that they can be extrated with organic solvent. Chromatographic methods will be extensively employed for separation and characterization of the metabolites. Gas chromatography will be used primarily for the hydrocarbon metabolites and the more volatile intermediate meterbolites normally associated with cellular growth. Paper column and thin layer chromatography will be employed in areas that gas chromatography is not applicable.

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III) Photodynamic Study. Bacterial cell suspension in the presence of testing hydrocarbon will be exposed to ultraviolet light and the total viable count will be estimated before and after exposure to the light. Per cent survival will be calculated on the basis of viable cell before the exposure. Comparison on per cent survival will be made for different testing hydrocarbons at different length of exposure.

IV) Morphological and Cytochemical Studies. Morphological and cytochemical studies will be made by staining technique for differentiation of shape and cytochemical changes. Qualitative changes of the cells caused by the carcinogens will be evaluated.

C. Significance of Research

1) Several relatively simple microbial techniques for assaying air pollutants for carcinogenic activity have already been developed. Although each technique fails for a number of test compounds there is an excellent possibility that a number of simple systematic tests might be combined into a broad screening assay technique. Thus the microbial techniques appear promising as alternates to the conventional animal tests.

2) An evaluation of degree of carcinogenic activity among isomers, with close environmental control, can be readily made using the microbial systems.

3) The biochemical approach of the present proposal involves fundamental studies on enzymatic activities of bacterial cells treated with carcinogenic hydrocarbons. The induced atypical bacterial cells may be comparable to cancer cells. Therefore the studies may lead to an insight of the chemical carcinogenesis in higher animals.

4) There is potential possibility of modifying or using present microbial systems for screening antitumor agents.

D. Facilities Available

Existing and fully equipped and staffed laboratories are available within the Sanitary Engineering Research Laboratory, University of California, Berkeley. Library and consultants of the highest quality are readily available.

Specific equipment currently available and in use includes incubators, ovens, autoclaves, balance, microscope, refrigerated centrifuge, sonic oscillators, spectrophotometers, Warburg's apparatus in addition to minor equipment. A low temperature incubator, vacuum oven and certain chromatographic equipment are currently being used on a short loan basis.

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2. SUPPORTING DATA

A. Previous Work

This laboratory has been engaged in the field of air pollution for a period of over ten years resulting in numerous publications concerned with the identification and resolution of atmospheric pollutants. Interest in assaying many of the resolved, unidentified compounds for carcinogenic activity was aroused in the early work. For example the clear resolution of an atmospheric sample by means of chromatography led to the ultimate identification of about 20 per cent of the compounds with many of these corresponding to known carcinogens. Many of the chromatogram peaks adjacent to the carcinogens could not and still have not been identified. Should an extended effort be made toward a more complete analysis on the basis of potential carcinogenecity? It became apparent that more sensitive assay techniques were needed for carcinogenic activity.

Previous work in this laboratory on developing more sensitive assay techniques involved the use of plant seedlings as the assay organisms introducing the test compounds hydroponically. The results were presented at a Symposium on Air Pollution sponsored by the American Chemical Society in 1963 but never published. The results were promising in that all compounds behaved as expected but the experiment was considered not satisfactory because over 60 days was required for the test.

Parallel work with bacteria appeared more promising and a publication eventually resulted.[8,9] Copies are being sent under separate cover. An aerobic organism Bacillus megaterium, was used in this work. Considerable effort was made at normalization and standardization of the technique but again the results were not completely satisfactory. A slight decrease in the dissolved oxygen had a comparable effect on the growth rate compared to the inhibitory effect of the carcinogens. A discussion of this effect is included under pertinent literature references (Section 2. C.).

Effort was shifted toward another organism, Phtobactrium phosphorium, a facultative anaerobe. The amount of dissolved oxygen was not limiting toward the growth. (A publication covering this research is currently being prepared and a rough draft is being sent under separate cover.) On this work the respiration rate was found to be indicative.

Another aspect considered under current work is the solubility of the test material in water. The low solubility of aromatic hydrocarbons in water might minimize the carcinogenic effect. To evaluate enhanced solubility a chemically defined medium plus glucose and 5 per cent acetone was inoculated with sewage. Three organisms were isolated of which one is currently being investigated. Relatively large amounts of carcinogen may be introduced in the acetone.

Biochemical, enzymatic and growth studies comparable to the herein described techniques are currently under investigation.

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B. Personal Publications

"Aromatic Hydrocarbons Produced From Simple Aliphatic Fuels," Mukai, M., Thomas, J. F., Tebbens, B. D., Anal. Chemistry, 37, 398 (1965).

"Particulate Air Pollutants Resulting From Combustion," Tebbens, B. D., Thomas, J. F., Mukai, M., ASTM Special Tech. Publication No. 352 (1964).

"Multidimensional Chromatography of Arenes Produced During Combustion," Mukai, M., Tebbens, B. D., Thomas, J. F., Anal. Chem., 36, 1126 (1964).

"Fluorescent Spectra of Aromatic Hydrocarbons Found in Polluted Atmospheres," Thomas, J. F., Tebbens, B. D., Sanborn, E. N., Crippa, J. M., Int. J. of Air Pollution, 2, 210 (1960).

C. Pertinent Literature References

The present investigation is primarily interested in the effect of chemical carcinogens on microbial cells. A summary of pertinent literature has been organized into 5 categories and is discussed below.

Point 1: Photodynamic action of carcinogens on unicellular paramecium has been extensively studied in recent years.[10] Although the exact nature of the mechanism has not been uncovered, the system has received considerable attention serving as a practical method for detecting carcinogens. The possibility of using bacteria as photodynamic assay organisms has been recognized using UV light on Saccharomyces carlbergensis in the presence of benzo(a)pyrene.[11] Recently Beebe and Pirsch[12] reported that Pasteurella species were killed at a rate proportional to the light intensity. The role of carcinogens in photodynamic action of Paramecium has been proposed as a sensitizing agent; tests of Pasteurella in similar conditions may also provide a useful indicator for the carcinogenic activity.

Point 2: According to "colonal selection theory,"[13] a carcinogen is considered to be capable of alternating the cellular environment so that the preexisting cellular variants are stimulated or permitted to grow. There is no direct biochemical pathway between the carcinogens and the hereditary information. A review of the literature has indicated a general pattern of microbial growth response toward chemical carcinogens. A summary is presented in Table 2. The growth of facultative anaerobic organisms is either stimulated or not affected by carcinogenic hydrocarbons. In contrast aerobic organisms are always inhibited by the carcinogens. Research in this laboratory also supports this conclusion.[8,9]

Point 3: The "instructional theory"[13] considers a carcinogen as a mutagen which participates directly in cytochemical alternation of the cells and eventually leads to irreversible change in hereditary informational content of the affected cell. Evidence of interaction between carcinogen and nucleic acid has been demonstrated in animals.[14,15] Nucleic acid in bacterial cells are also affected in the polynuclear carcinogenic hydrocarbon. The ratio of RNA/DNA in Escherichia coli treated with the carcinogen was shown to be lower than cells treated with non-carcinogens.[16] Since DNA constitutes genes, the effect of the carcinogen on nucleic acid may lead

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TABLE 2. EFFECT OF CARCINOGENS ON MICROORGANISMS BASED ON THE GROWTH RATE AND ON REACTION TO OXYGEN

Growth[1]	Tested Organisms	Relation to Oxygen	Carcinogens Tested	Dosage ug/medium	Incubation Period	Degree of Effect	References
Inhibition	Bacillus megaterium	aerobic	benz(a)pyrene	0.2 ug/ml	20 hr.	---	Applied Microbiology 10:217-222, 1962
	Lactobacillus casei	microaerophic	p-amino methyl amino azobenzene	1-3 ug/ml	---	---	Cancer Research 7:730, 1947
	Chaetomium sp. (mushroom)	aerobic	dibonz(a,h) anthracene	20 ug/ml	2 days	---	Growth 9:177-187, 1945
	Fusarium lini	aerobic	dibenz(a,h) anthracene	saturated solution of u.v. exposed compound 1 ml/100 ml	5 days	---	Proc. Soc. Exp. Biology and Medicine 38: 370-372, 1938
	Vibrio choleae	aerobic	na-salts of dibenz(a,h) anthracene and 20-methyl cholanthrene	32 ug/ml	20 hrs.	---	J. Infectious Disease 89: 59-75, 1951
	Streptococcus pyogenes	facultative anaerobic					
	Streptococcus salivarius						
	Staphylococcus aureus						
	Corynebacterium diphtheria						
Stimulation	Escherichia communion	facultative[3]	dibenz(a,h) anthracene and 3-methyl cholanthrene	300 ug/ml	9 hr.	50%	Science 86: 176, 1937
	Escherichia communion	facultative	dibenz(a,h) anthracene	---	9 hr.	---	J. Bacteriol. 38:13-23, 1939
	Escherichia coli	facultative	dibenz(a,h) anthracene and 3-methyl cholanthrene	water saturated solution	---	19-36%	J. Bacteriol. 44:394, 1942
	Staphylococcus aureus	facultative					

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Table 2 (cont'd)

Growth[1]	Tested Organisms	Relation to Oxygen	Carcinogens Tested	Dosage ug/medium	Incubation Period	Degree of Effect	References
Stimulation	Saccharomyces ellipsoideus	facultative	3-methyl cholanthrene	32 ug/ml	---	---	Ann. Missouri Bot. Garden 24:583-589, 1937
No Effect	Escherichia coli	facultative				0	J. Nat. Cancer Inst. 10:11-27, 1949
	Saccharomyces ellipsoidous	facultative				0	Ann. Missouri Bot. Garden 28:1-30, 1941

[1]Growth compared to the control treatment

[2]Both carcinogens tested were in the forms of Na-Salts which could behave differently from the original carcinogens

[3]Facultative anaerobic organisms

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to mutation of the cell. The mutaganic nature of the carcinogen has been tested by susceptibility of the E. coli to bacterial phage. Non-carcinogens do not show any mutagenic properties.[17] Prolonged exposure of various bacterial cells to polynuclear carcinogenic hydrocarbons has induced changes in morphology and growth rate.[18,19]

Both the "colonal selection" and "instructional" theories eventually deal with heterogenic populations predominanted with variants or mutants which in the pathological sense are equivalent to tumor cells in the higher animal.

Point 4: Although it is commonly accepted that molecular structure of a protein is a literal translation of DNA molecules, yet the presence of a given DNA molecule in a cell does not always produce the corresponding enzyme(s) because the gene expression is controlled by feedback systems, i.e., "negative feedback" and "repression." From a biochemical viewpoint, uncontrollable proliferation of cancer cells signifies a possible upset in metabolic regulation of the cellular activities. A general practice of pathological studies is to compare enzymatic activities in diseased cells and in normal cells. Such examination is made to reveal the failure of enzyme forming systems as well as the inactivation of certain enzymes associated with the disease. In the animal system cancer cells can be induced by aromatic hydrocarbons which were demonstrated to interact with the cellular protein.[20] Histological studies in rat tissue have revealed that the carcinogens may affect the Sulfhydryl group of the protein in tissues.[21] Since sulfhydryl groups constitute the active groups in some enzymes, question is naturally raised whether carcinogenic hydrocarbon can specifically react to inactivate sulfhydryl group of the enzyme or can cause alternative metabolic pathways by changing the enzyme structure resulting in "enzyme delation."[22] Information concerning inactivation of enzymes containing active sulfhydryl groups, such as lactic dehydrogenase in muscle[23] and alternation of metabolic pathways in animal tumor cells[24] has been reported. Corresponding studies on microorganism has been very limited. Some preliminary biochemical studies on the effect of carcinogenic aromatic hydrocarbons on Bacillus megaterium have been reported.[9] The results indicate that the hydrocarbons affect the dehydrogenase as shown by methylene blue reduction. The organism also accumulates lactic acid in the cells treated with carcinogens. It is hoped that the interaction between carcinogenic hydrocarbons and the cellular components in the microbial system can be shown and the resulting effect may reveal qualitative and quantitative aberration of an enzyme activity (or activities). The enzymatic aberrations caused by the carcinogens in the microbial system may eventually serve as a model for the studies in animal carcinogenesis.

Point 5: Studies in liver microsome in recent years have indicated that the activity of TPNH-dependent enzymes can be markedly influenced by carcinogenic hydrocarbon such as benz(a)pyrene.[25] The carcinogen stimulates the synthesize of the enzymes. Similar studies on microbial system have not yet been done. Likewise, some of the highly active enzymes associated with cancer cells in animals have not yet been evaluated in the microbial system treated with carcinogens. This is a field of great promise in cancer research, because a relationship between tumors of higher organisms and equivalent "tumors" in the microorganism may be linked through this line of approach. Investigation in this direction may yield data of comparative physiological significance to the higher form.

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References

1. Lowry, O. H., N. J. Rosebrought, A. H. Farr and R. J. Randall, "Protein measurement with Folin phenol reagent." Journal of Biological Chemistry, 193:265-275, 1951.

2. Weber, G., "Comparison of enzyme activities in hepatomas of different growth rates," Proceedings, American Association of Cancer Research, 3:286, 1962.

3. Neilands, J. B. and P. K. Stumpf, Outline of Enzyme Chemistry, Wiley, New York.

4. Pitot, H. C. et al., "Transaminase activities of liver tumors and serum," Cancer Research, 21:1522-1531, 1961.

5. Rechcigl, M., Jr., V. E. Price and H. P. Morris, "Studies on the catalase activity of various hepatomas," Proceedings, American Association for Cancer Research, 3:232, 1961.

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SECTION B - PRIVILEGED COMMUNICATION

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3. BIOGRAPHICAL SKETCHES

(Give the following information for EACH key staff member, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	DATE OF BIRTH (Mo. Day Yr.)
Jerome F. Thomas	Professor of Sanitary Engineering	REDACTED
PLACE OF BIRTH (City, State, Country)	PRESENT NATIONALITY (If non-U.S. citizen, indicate visa status)	SEX
Chicago, Illinois	USA	☒ Male ☐ Female

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED
DePaul University, Chicago, Illinois	B.S. (Chem)	1943
University of California, Berkeley	Ph. D. (Chem)	1950

HONORS

MAJOR RESEARCH INTEREST

Research in area of water pollution
Research in area of air pollution

RELATIONSHIP TO PROPOSED PROJECT

Principal Investigator

RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Starting with present position, list ALL experience relevant to project)

Actively engaged in teaching and research in the field of air and water pollution for the last 15 years. Between 40 and 50 research papers have been published in scientific journals. Faculty Investigator for numerous master's and doctorate candidates in the field of sanitary engineering (chemical aspects of air and water).

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SECTION II -- PRIVILEGED COMMUNICATION
Continuation page AP 00469-01

Name: Chi-Sin Chen Title: Assistant Research Microbiologist

REDACTED

Place of Birth: Li-Kang, Taiwan, China

Visa Status: H-1 visa (3 year), Distinguished Visitor Sex: Male

Education: National Taiwan University, Taipei, Taiwan, China, B.S., 1957
Agricultural and Mechanical University of Texas, College Station, Texas, M.S., 1962
Oregon State University, Corvallis, Oregon, Ph.D., 1965

Major Research Interest: Studies on microbial physiology with special interest in enzymatic reactions. Research work on microbial biochemistry.

Relationship to Proposed Project: Direct and perform major bench aspects of proposed work

Research and/or Professional Experience:

Full time research for past 8 months on the project related to the present proposed work. Work on Master's and Doctorate thesis.

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